

RESPIRATION AND ANAEROBIC SURVIVAL IN SOME SEA WEED-INHABITING INVERTEBRATES^{1, 2}

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L. C. Beadle in a paper on the respiration in the African swampworm *Alma emini* (Beadle, 1957) showed that the waterlogged mats of *Cyperus papyrus* are devoid of oxygen most of the time and that all animals living in them have to be adapted to this situation one way or the other. The case of the swamps is certainly an extreme one, but there can be no doubt that wherever plants grow in water or are easily flooded, anaerobic conditions may prevail occasionally or quite regularly.

The large sea weeds growing along the open coasts seem to represent an exception to such a statement and might be regarded as the prototype of a well-aerated habitat. This is certainly true at high tide. At low tide, however, these sea weeds collapse, forming thick clumps within and under which water is trapped. In the night or on cloudy days respiration of the algae may exceed photosynthesis and the water, cut off from atmospheric circulation, will gradually lose its oxygen to the plants.

This, then poses an ecological problem which can be stated as follows: Does the oxygen content of the water trapped by emersed sea weeds ever fall to zero and if so, how do the animals living in the sea weeds cope with this situation?

MATERIAL AND METHODS

The work was carried out at the Woods Hole Oceanographic Institution, Woods Hole, Massachusetts. The water samples were taken from under clumps of emersed brown algae (*Fucus*, *Ascophyllum*) on July 13 and 14, 1959, by means of 5-cc. syringes which were stoppered with glass plugs, and analyzed in the laboratory with the gasometric water analyzer of Scholander (Scholander *et al.*, 1955). The sampling site was a flat piece of gravelly and rocky beach (Woods Hole Yacht Club) on which brown algae grew quite luxuriantly. All the samples came from the upper and the middle zone of the exposed algal belt. The tidal range at Woods Hole is 1.8–2.2 ft. The temperature within a clump of sea weeds measured 20° C. at one occasion.

Various animals living in the large brown sea weeds around the Woods Hole Oceanographic Institution were collected at high tide and their respiration and anaerobic survival studied. The respiration of nematodes and mites was measured by the Cartesian diver method as described by Holter and Linderstrøm-Lang (1943) and others. The divers used had a volume from 6.5 to 37.4 μ l. and volume changes could be read to 0.001–0.006 μ l. O₂/hr. The respiration of the

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amphipods was measured by means of the volumetric micro- and macro-respirometer of Scholander (Scholander *et al.*, 1952). Respirometer runs were made at 10° C. and in the following diagram the respiration values are corrected to 20° C., the temperature at which the diver was run, by assuming a Q_{10} of 2. This value seems to be a reasonable—perhaps even too low—guess in the light of the data on the amphipod *Talorchestia* by Edwards and Irving (1943) as re-plotted by Rao and Bullock (1954).

Anaerobic survival was studied by putting the animals into a small amount of water in a Thunberg tube which then was evacuated and flushed with tank-nitrogen. Constant experimental conditions were maintained by putting a constant amount

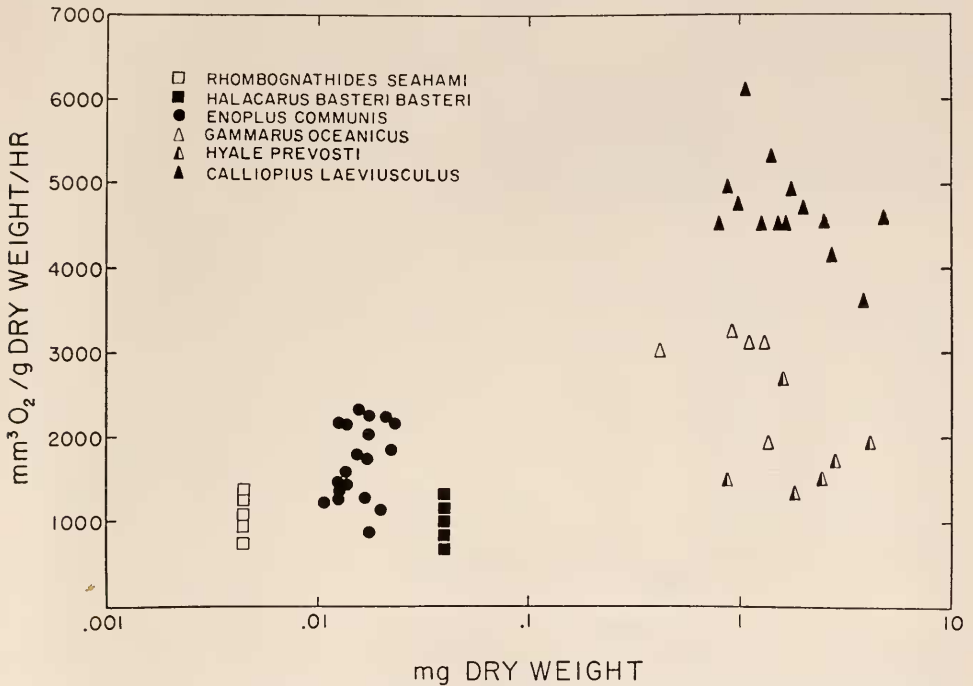


FIGURE 1. Oxygen consumption per gram dry weight of the six species of animals investigated. Each convention represents one experiment.

of water into the Thunberg tubes and by flushing each tube exactly 100 times. The evacuation of the Thunberg tubes was carried out at room temperature. Subsequently, the tubes were kept either in the refrigerator at approximately 2° C., or at 15° C., or in the laboratory which had a fairly constant temperature of 25° C. The behavior of the animals was studied either by observing them in the closed tube, or after a certain time had elapsed, by emptying them into a dish with normal sea water. The recovery time of nematodes and mites was defined as the time interval within which 75% of the animals used for one experiment had resumed movement.

The dry weight of the nematodes was established by measuring volume and specific gravity as described elsewhere (Wieser, 1960). The dry/wet weight ratio

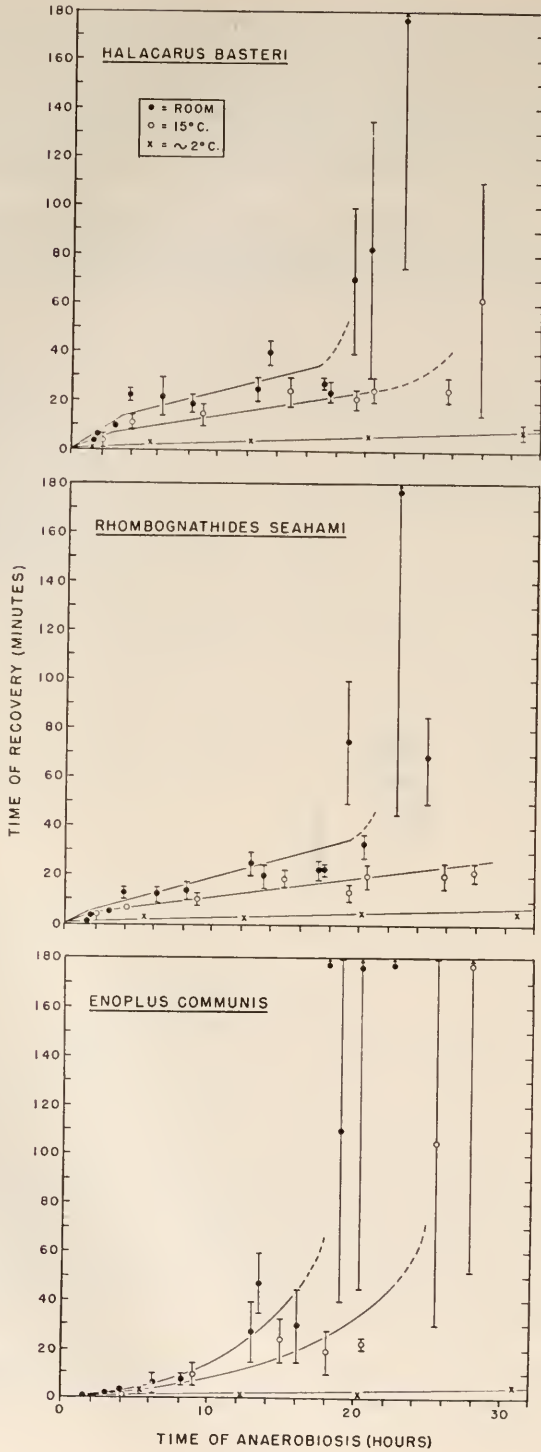


FIGURE 2.

was assumed to be 25% in all specimens. The mites were dried at 100° C. and weighed in batches on a "Misco" quartz helix (by Dr. R. Conover, W.H.O.I.). The dried amphipods were weighed individually on a torsion balance.

RESULTS

The water analyses gave the following results:

Sample no.:	1	2	3	4	5	6
Time:	10 P.M.		10 A.M.			
Ml. O ₂ /1 liter H ₂ O:	0.25	0.30	0.0	0.05	0.96	1.65

The night samples were taken exactly at low tide, the day samples 30–40 minutes after low tide on a cloudy day (illumination 1700 foot-candles). The oxygen content of saturated sea water at 20° C. and the local salinity (approximately 31‰) is 5.5 ml. O₂/1 liter H₂O (Sverdrup *et al.*, 1946).

For the measurement of respiration and anaerobic survival two groups of species were chosen; one, consisting of the nematode *Enoplus communis* and the sea mites (Halacaridae) *Rhombognathides seahami* and *Halacarus basteri basteri*, represented very small animals of low motility, unable to swim and without special respiratory organs. The other group consisted of three amphipod species, *Calliopius laeviusculus*, *Gammarus oceanicus* (identified by Dr. H. Werntz, Harvard University) and *Hyale prevosti*, representing medium-sized, very agile animals with good swimming capabilities and equipped with gills.

The respiratory rates per gram dry weight of the six species investigated are summarized in Figure 1. It is evident that if the size difference is taken into account (Zeuthen, 1947), the amphipods have a much higher metabolic rate than the other three species.

The two groups of species could also be distinguished in their reaction to anaerobic stress. Nematodes and mites, after the oxygen had been removed from the Thunberg tube, became paralyzed. From this state they recovered after they were put back into oxygenated water, provided that the time of anaerobic stress had not lasted too long. The recovery time as shown in Figure 2 is a function of the time of anaerobiosis and of the temperature at which the latter was spent. The three amphipods, however, remained active under anaerobic stress for a certain time and then died (Fig. 3). One could, of course, say that they, too, became paralyzed after a period of activity, but that the paralyzed state which preceded death was of extremely short duration. If an animal after readmission to normal conditions did not recover right away, it did not recover at all.

FIGURE 2. Relationship between time of anaerobiosis and time of recovery in the two halacarids (upper two diagrams) and the nematode. Each convention represents one experiment on a batch of animals. Range of variability in recovery time indicates beginning of movements in the first and in 75% of the animals in one batch. Recovery periods exceeding 180 minutes are characterized by an arrow and by a circle close to the upper abscissa of the diagram.

The survival period of one amphipod, *Calliopius laeviusculus*, was extremely short, certainly not more than 5 minutes at a temperature of 25° C. The other two species, *Gammarus oceanicus* and *Hyale prevosti*, did considerably better, the survival period ranging from about half an hour of anaerobic stress to about three hours. There was no clear-cut relationship between survival time and the size of the animals.

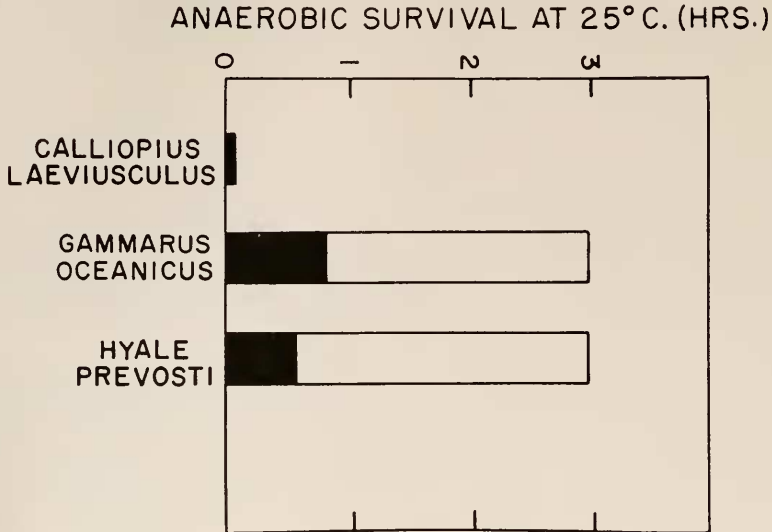


FIGURE 3. Survival under anaerobic conditions of the three species of amphipods studied. The black part of each column indicates the time of anaerobiosis all individuals survived, the white part indicates ranges of variability of survival time.

DISCUSSION

The water analyses show plainly that animals living in the water trapped by large sea weeds at low tide may be subject to oxygen-free or almost oxygen-free conditions. Under extreme circumstances (very large clumps of algae, no light, high temperature) these conditions probably set in soon after the receding tide has uncovered the plants and may last for several hours. A drop in the oxygen content to 30% saturation value was found by Revelle and Emery (1958) in intertidal basins with algal growth at the bottom.

Animals living in sea weeds react in different ways to the movements of the tides. As shown previously (Wieser, 1952), some animals remain in the sea weeds all the time while others (in Plymouth, England, *e.g.* copepods and the amphipod *Stenothoe monoculoides*) leave their habitat with the receding tide and return to it with the rising tide. To the former group, obviously, belong slow-moving, non-swimming animals like the nematode and the mites studied in this paper. These species are adjusted to their environment by being able to withstand anaerobic conditions at 25° C. for at least 12 hours which, in an area with diurnal tides, is the theoretical maximum their habitat could remain emersed.

That paralysis during anaerobiosis is due to the accumulation of toxic metabolites within the body has been pointed out by von Brand (1946). Since the accumulation of metabolic substances is a temperature-dependent process, the close dependence of the rate of recovery from paralysis on the temperature at which the anaerobic period was spent (Fig. 2) could have been anticipated (see also Miller, 1957). Furthermore, the recovery time from anaerobiosis is a characteristic of each species. In the two halacarids, a linear relationship exists between the time of anaerobiosis and the time of recovery, up to the point at which the effects of asphyxiation become lethal and the curve expressing the relationship turns sharply upwards (Fig. 2). The slope of the linear portion of the curve is steep in the beginning and flattens out somewhat after 2-4 hours' application of anaerobic stress at room temperature. The points of inflection of the triphasic curve and the absolute values of recovery time, but not the slopes of the curves, are different in the two species of Halacaridae. However, the triphasic curve is well defined only for the experiments at room temperature. The 10-times larger species of *Halacarus* is more susceptible to the effects of asphyxiation than the species of *Rhombognathides*.

The relationship between time of anaerobiosis and time of recovery in the nematode *Enoplus communis* is an exponential one.

Kalmus (1942), in similar experiments, reported the recovery time to be different in different species and even mutants of *Drosophila*. In this genus, as in the halacarids over a certain period, a linear relationship between time of anaerobiosis and time of recovery was observed.

The second group of species, which consists of animals obviously able to leave and to repopulate the sea weeds at will, does not have to be adjusted for survival of anaerobic periods. The efficiency of receptor and locomotor organs of amphipods and related forms can always be considered as being sufficient to keep the animals in oxygenated water all the time. Accordingly *C. laeviusculus* has almost no resistance to the reduction of oxygen in its environment. The data in Figure 3 show that even five minutes of anaerobic conditions bring about the death of all members of this species, but the other two species of amphipods show some resistance. A related species of *Gammarus*, *G. duebeni*, is even known to occur on European coasts in rock pools and stagnant waters smelling of H_2S (Kinne, 1959).

The data of Figure 1 show that the least resistant amphipod, *C. laeviusculus*, has twice the oxygen consumption of the other two amphipod species. This might reflect the fact that the former species also leads a pelagic life, particularly in winter (Kunkel, 1918).

In a very general way it can be concluded that the nematode and the two halacarids in an anaerobic environment produce and deposit metabolic breakdown products which are toxic and cause paralysis. Up to a certain time, however, the tissues are not irreversibly injured by these breakdown products. The failure of *Calliopius laeviusculus* to endure even very short periods of de-oxygenation might be due either to its inability to gain sufficient energy under anaerobic conditions, or to the irreversibly harmful effects of even very small amounts of the breakdown products of anaerobic metabolism. The behavior of the remaining two amphipod species could be explained by assuming that either their energy or their excretory mechanisms function imperfectly under anaerobic conditions.

SUMMARY

1. The water trapped by large brown sea weeds at low tide may become oxygen-free in the night or on cloudy days.

2. Slow-moving, non-swimming animals living in the sea weeds all the time (like nematodes and mites) are paralyzed by the removal of oxygen from the water, but they recover from this state if the period of anaerobic stress has lasted less than approximately 16 hours at 25° C. The relationship between time of anaerobiosis and time of recovery is temperature-dependent and a characteristic of each of the three species investigated (*Enoplus communis*, *Rhombognathides seahami*, *Halacarus basteri basteri*).

3. Agile animals, capable of leaving and repopulating the sea weeds with the tides, show a different reaction pattern. One of the amphipods investigated (*Calliopius laeviusculus*) did not survive even a few minutes of anaerobiosis, while the other two species (*Gammarus oceanicus*, *Hyale prevosti*) survived from about half an hour to three hours of anaerobiosis. The former species occasionally leads a pelagic life and has twice the respiratory rate of the latter two species.

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